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BRIEF COMMUNICATION

ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES OBTAINED BY GREEN SYNTHESIS

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SUMMARY

Silver nanoparticles (AgNPs) are metal structures at the nanoscale. AgNPs have exhibited antimicrobial activities against fungi and bacteria; however synthesis of AgNPs can generate toxic waste during the reaction process. Accordingly, new routes using non-toxic compounds have been researched. The proposal of the present study was to synthesize AgNPs using ribose as a reducing agent and sodium dodecyl sulfate (SDS) as a stabilizer. The antifungal activity of these particles against *C. albicans* and *C. tropicalis* was also evaluated. Stable nanoparticles 12.5 ± 4.9 nm (mean ± SD) in size were obtained, which showed high activity against *Candida* spp. and could represent an alternative for fungal infection treatment.

KEYWORDS: Silver nanoparticles; Antifungal activity; *Candida* spp.

Candida albicans and *Candida tropicalis* yeasts are responsible for a number of major diseases as well as recent cases of resistance to the main antifungals. Therefore, new substances should be researched as an alternative to combat such resistance[4,11]. *C. albicans* and *C. tropicalis* are the main yeasts isolated from samples of patients admitted to hospitals in the state of Ceará, Brazil[12].

Nanotechnology is responsible for the production and study of metal nanoparticles. These structures present several applications that highlight antimicrobial activity, and this property is an important tool in combating microorganisms resistant to conventional drugs[18].

Silver nanoparticles (AgNPs) are a new kind of material with several applications, such as sensors, catalysts, anticancer agents and antimicrobial agents. AgNPs have exhibited activity against bacteria, fungi and viruses[8]. However, synthesis of AgNPs produces toxic waste, such as ammonia[14], which can affect human health and the environment[18]. The green synthesis of AgNPs has used various routes: plants, microorganisms and non-toxic substances[6,15,19].

The aim of this study was to synthesize AgNPs using ribose sugars as reducing agents and sodium dodecyl sulfate (SDS) as the capping agent. The antifungal activity of these nanoparticles was evaluated against strains of *C. albicans* and *C. tropicalis*.

The synthesis of AgNPs was performed using ribose (Sigma-USA). First, 500 mL of a 5 mM AgNO3 (Merck-Brazil) solution was added to 1.0 g of ribose and 0.5 g of SDS (Sigma-USA) was used as the stabilizer. This solution was stirred and the temperature was raised to 50 °C. SDS had the function of preventing agglomeration and subsequent precipitation of the AgNPs. The reaction was considered complete when the solution acquired a pale yellow color, characteristic of AgNPs (Fig. 1a)[2,14].

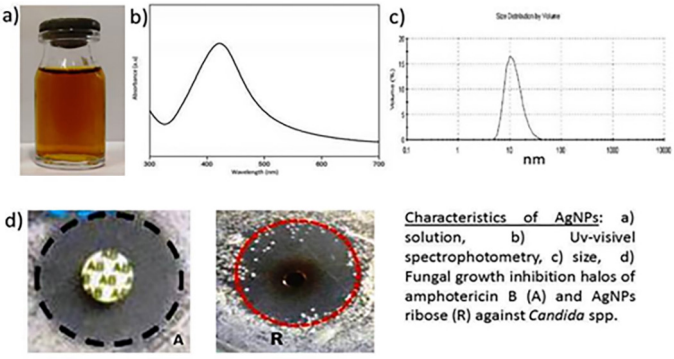

Fig. 1 - Characterization of the AgNPs and their antifungal effects.

The purification was carried out by centrifugation at 10,000g/10min. Characterization of the synthesized AgNPs was carried out using a UV-Visible spectrophotometer (Thermo Scientific GENESYS™ 10s), by scanning of the absorbance spectra in a 300-700 nm range of wavelength.

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Table 1
Effect of AgNPs produced by green synthesis and Amphotericin B against *C. albicans* and *C. tropicalis*

Strains (*n*)	AgNPs		Amphotericin B		*p*
	Range (mm)	Halo (mm) (Mean ± SD)	Range (mm)	Halo (mm) (Mean ± SD)	
C. albicans (14)	17-30	23 ± 4	15-25	20 ± 3	
C. tropicalis (16)	12-30	21 ± 4	15-25	20 ± 3	0.02

The size of the AgNPs was analyzed on Zetasizer, NanoZS Malvern® by dynamic light scattering (DLS)[5].

In this study, 30 strains of *Candida* spp. were selected (14 *C. albicans* and 16 *C. tropicalis*) and isolated from blood samples of patients hospitalized in the state of Ceará, Brazil. *C. albicans* was purified and identified in a chromogenic medium, with production of chlamydospores in rice extract agar containing Tween-80, and germ tube formation. This identification was carried out by molecular biology with the primer *hwp*1 (cr-f-5'-GCT ACC ACT TCA GAA TCA TCA TC-3'; cr-r-5' GCA CCT TCA GTC GTA GAG ACG-3') and the PCR conditions were 95 °C for five min, followed by 30 cycles of 94 °C for 45 s, 58 °C for 40 s, and 72 °C for 55 s; extension was performed at 72 °C for 10 min. The DNA fragment size that was produced had 945 bp. The molecular identification of *C. tropicalis* was performed using the *trf*4 gene. The following primers were used (trf4 5'-ATT GGC TGA AAC AGA GGT-3 '; trf4-5' CAA CCC TGC TAA GTC ATT AC-3') and the PCR conditions were 95 °C for five min, followed by 30 cycles of 94 °C for one min, 50 °C for one min, and 72 °C for 90 s; extension was performed at 72 °C for 10 min. The DNA fragment size that was produced had 324 bp[7,16].

The sensitivity of *Candida* spp. was evaluated by the well diffusion method on a Mueller-Hinton medium supplemented with 2% glucose and 0.05% methylene blue. In mediums containing *Candida* spp, wells were made and filled with 80 µg of AgNPs. Discs of amphotericin B 10 µg were used as control. The plates were incubated at 35 °C for 24h, and after this period fungal growth inhibition halos were measured (mm). Each test was conducted three times, according to the protocol of CLSI M44-A2[3,20].

Production of AgNPs using ribose as a reducing agent and SDS as a capping agent was simple and easy to perform. The entire process was completed in 30 min. The chemical reaction proposed can be represented by the following equation:

$$C_5H_{10}O_5 + 2Ag^+ \rightarrow C_5H_8O_5 + 2H^+ + 2Ag^0\,(AgNPs)^{(1)}$$

These AgNPs showed strong spectrophotometric absorbance, around 420 nm, as shown in Figure 1b. This is typical behavior of these structures. Use of SDS as the capping agent provided prolonged stability for up to four months when stored at room temperature and exposed to ambient light. Some sugars have reducing properties and are used in the production of nanoparticles. The process does not harm the environment because it does not produce toxic waste and requires no accelerator[13].

AgNPs produced in this study had a size of 12.5 ± 4.9 nm (mean ± SD), with a narrow particle size distribution, as shown in Figure 1c. This feature gives a high surface area, better for antimicrobial activity and good order. In previous studies using glucose as the reducing agent, the size of AgNPs was around 15 nm[10].

The AgNPs exhibited high antimicrobial activity, and this property can be very useful, especially against microorganisms resistant to conventional antimicrobials[17]. *C. albicans* and *C. tropicalis* showed high sensitivity to AgNPs (Fig 1d). The activity of 80 µg of AgNPs can be compared with the activity of amphotericin B, a powerful antifungal (Table 1). Studies highlight this same result with activity of AgNPs against *Candida* spp[9,14]. The statistical analysis of the results, carried out by Student's t-test, showed that *C. albicans* was more sensitive than *C. tropicalis* ($p = 0.02$).

In conclusion, AgNPs were easily prepared by green synthesis using ribose as a reducing agent and SDS as a stabilizer. Additionally, they showed high activity against *C. albicans* and *C. tropicalis*, a similar activity observed by the antifungal amphotericin B, and may represent an alternative for treating fungal infections.

RESUMO

Atividade antifúngica de nanopartículas de prata obtidas por síntese verde

Nanopartículas de Prata (AgNPs) são estruturas metálicas em escala nanométrica. AgNPs apresentam atividades antimicrobianas contra fungos e bactérias; no entanto, a síntese de AgNPs pode gerar resíduos tóxicos e devido a isso novas rotas utilizando compostos atóxicos têm sido buscadas. O objetivo desse estudo foi sintetizar AgNPs utilizando a ribose como agente redutor e dodecil sulfato de sódio (SDS) como estabilizante e avaliar a atividade antifúngica dessas partículas contra *C. albicans* e *C. tropicalis*. Foram sintetizadas nanopartículas estáveis com 12,5 ± 0,2 nm (média ± DP) que apresentaram elevada atividade contra *Candida* spp. e podem representar boa alternativa no tratamento de infecções fúngicas.

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